FRUCTOSAMINE (µmol/L)
MANUAL OR AUTOMATED METHOD
C412-0A; C414-02

Intended Use
For the ***in vitro*** diagnostic use in the quantitative determination of Fructosamine in serum.

Clinical Significance
Non-enzymatic glycation of blood proteins has been reported to occur through formation of ketoamines in a two step reaction: 1. Formation of a Schiff base by reversible coupling of glucose to protein. 2. Non-reversible Amadori rearrangement to the corresponding ketoamine or Fructosamine. The amount of Fructosamine in serum is increased in diabetes mellitus owing to the high concentration of glucose in blood. The concentration of Fructosamine in serum thus reflects the degree of glycemic control attained by diabetic patients and is useful in monitoring the effectiveness of therapy in diabetes over a period of several weeks, in a manner analogous to the determination of glycated hemoglobin. However, unlike glycated hemoglobin which represents the average blood glucose concentration over six to eight weeks; Fructosamine reflects the average glucose concentration over the previous two to three weeks. Thus the advantage is that Fructosamine responds more quickly to changes in therapy allowing for improved glycemic control. (1-3)

Method Principle
This kinetic test for fructosamine (glycated protein) is based on the ability of ketoamines to reduce nitro blue tetrazolium in alkaline conditions to form a purple colored formazan complex. By monitoring the intensity of the color produced at 530nm, the concentration of Fructosamine in the serum or plasma sample is determined (4-6)

Reagent
Each liter contains:

Buffer	
Cholic Acid	0.49 mmol
Nitroblue tetrazolium	0.57 mmol
Surfactant and nonreactive ingredients	

Precautions
Handle this reagent using good laboratory practice. **DO NOT PIPETTE REAGENT BY MOUTH.** Avoid contact with skin and eyes. If contact occurs, wash affected area with plenty of cold water. Clean spills immediately.

Reagent Storage and Stability
Store the Fructosamine reagent at 2-8°C. When stored as directed, the unopened reagent is stable until the expiration date stated on the label. Once opened, the reagent is stable for at least six months when stored at 2-8°C, and tightly capped between uses.

Reagent Preparation
Catachem's single fructosamine reagent is packaged ready to use. No preparation is required.

Indications of Reagent Deterioration
- Turbidity
- Absorbance > 0.5 OD, 1 cm light path, 530nm
- Quality control values out of assigned ranges

If these reagent characteristics are observed, call Catachem technical service.

Specimen Collection and Stability
Clear unhemolyzed sera are the specimens of choice. Serum should be separated immediately from the clot and analyze promptly or stored at 2-8°C. Serum Fructosamine is stable for 3 days at 18-26°C, two weeks at 2-8°C and two months frozen at -20°C.

Procedure
These instructions are outlined for performing the Catachem Fructosamine assay using the manual or automated method. Read the entire original instrument manufacturer's instructions procedure before performing this Fructosamine procedure.

Materials Provided
Catachem Fructosamine Reagent

Materials Required (But Not Provided)
- Analyzer equipped with 530nm wavelength
- Calibrator material with assigned Fructosamine value
- Catachem Quality control material with assigned Fructosamine values

Tests Parameters

Sample Volume	26 µl
Reagent Volume (R1)	500 µl
Reaction Temperature	37°C
Units	µmol/L

Calibration
It is recommended to use the Catachem Fructosamine Calibrator, containing a known Fructosamine value.

Calibration Schedule
Calibration should be performed when this method is first implemented. Recalibration is required after changes of reagent lot number, major instrument service, and when quality control values are out of the indicated range.

Calibration Procedure
Instructions for calibrating the automated analyzer are provided by the specific instrument manufacturer. Read the entire recommended calibration procedure before proceeding with the instrument calibration. Use Catachem Fructosamine Calibrator for the single point calibration.

Quality Control
To monitor the quality performance of the procedure used, Catachem Fructosamine Control Level I and Control Level II with assigned Fructosamine values should be included in the assay procedure each time the assay is run.

Directions for Use
The Catachem Fructosamine method requires a single reagent.

Assay Procedure
1. Label cuvettes or appropriate test tubes as: a) Calibrator (CAL), b) Control 1 (C1), c) Control 2 (C2), d) Sample (SAMP).
2. Start a timing clock and pipette the Catachem Fructosamine Reagent and sample volumes into the cuvettes or test tubes at fixed intervals as shown in the table below.
3. After all reaction components are in the cuvettes, quickly mix all cuvettes without delay.
4. Read the absorbance in each cuvette immediately after the 4 minute incubation period ends. Read each cuvette at the same fixed interval in which sample was introduced to reagent.
5. After exactly 5 minutes, i.e. a 1 minute read time for each cuvette, read all cuvettes again at the same fixed time interval as before and calculate Δ-absorbance between initial 4 minute read and final 5 minute read.
6. Proceed with calculation or results as shown below.

353 Christian Street Suite 2● Oxford, Connecticut ● 06478 USA ● Phone: (203) 262-0330 ● FAX: (203) 262-9836 ● email:catachem@catacheminc.com

	Calibrator	Control I	Control II	Sample
SAMPLE	**0.026 ml**	**0.026 ml**	**0.026 ml**	**0.026 ml**
REAGENT	**0.50 ml**	**0.50 ml**	**0.50 ml**	**0.50 ml**

Calculations and Results

$$\text{Fructosamine } \mu\text{mol/L} = \frac{\Delta\text{- Abs. Samp.}}{\Delta\text{- Abs. Cal.}} \times \text{Cal. } \mu\text{mol}$$

Example:

	Abs-1.	Abs-2.	Δ-Abs.
Sample	0.100	0.200	0.100
Calibrator	0.200	0.320	0.120

Calibrator assigned value = 491 µmol

$$\text{Samp Fructosamine (µmol l)} = \frac{0.100}{0.120} \times 491 \text{ µmol}$$

$$= 409 \text{ µmol}$$

Interfering Substances

The following substances have no significant effect on the accuracy of this Fructosamine procedure at the concentrations stated.

- Hemoglobin ≤ 100 mg/dL
- Triglycerides ≤ 1200 mg/dL
- Bilirubin ≤ 4.0 mg/dL

Other substances and certain drugs are also known to influence the Fructosamine values. (7).

Expected Values (5,6) 205-285 µmol/L

The normal range was obtained from a study of 555 ambulatory, non-diabetic, healthy human subjects. The group included an equal number of males and females ranging between the ages of 20 and 60 years old. Hemolytic and icteric sera were excluded from this study.

The values given here are only to be used as a guideline. It is recommended that each laboratory establish the normal range for the geographical area in which it is located (8).

Method Performance Characteristics

Sensitivity: Using a pathlength of 1 cm, a Δ-absorbance of 0.04 mA per µmo/l should be obtained.

Linearity: This procedure is linear over the range of 10-1000 µmol/L.

Procedure Limitations

Samples with Fructosamine values greater than 1000 µmol/L should be diluted 1:2 with physiological saline and reassayed. Multiply results obtained by 2 to adjust for the sample dilution.

Precision: Precision data was obtained using three levels of human serum samples and following the NCCLS EP5-T2 procedure (9). The following results were observed.

Precision

Fruct.	Within-Run		Day-Day		Total	
Mean	SD	CV	SD	CV	SD	CV
µmo/L	µmo/L	%	µmol/L	%	µmol/L	%
215	9.6	4.45	15.9	8.04	15.98	7.76
513	14.29	2.78	22.37	4.68	25.91	5.23
830	19.34	2.78	27.17	3.44	30.81	3.81

Accuracy

Using an automated analyzer, correlation studies were carried out between this Fructosamine procedure (Y) and a commercially available Fructosamine test procedure as reference (X). Serum samples were assayed and the results compared by the least squares regression. The following statistics were observed:

N = 40
Range = 60-980
Mean Y = 416.9
Mean X = 385.7
Y = 1.06 X + 8.55
Sy.x = 26.82
r = 0.995

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353 Christian Street Suite 2● Oxford, Connecticut ● 06478 USA ● Phone: (203) 262-0330 ● FAX: (203) 262-9836 ● email:catachem@catacheminc.com